With the Author's compliments.

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A note on the histology of sterile incubated cancerous and healthy tissues.[1]

By Samuel G. Shattock and Charles A. Ballance.

[With Plates XXXIV and XXXV.]

In our previous communication we were able to show that portions of cancer (*i. e.* carcinoma or sarcoma) and portions of normal tissues, viz. muscle, kidney, testicle, liver, submaxillary salivary gland, could be maintained sterile upon blood-serum, agar-agar, and in nutrient jelly for an indefinite period at a temperature of 100° F. Some of the tubes which we had the honour of submitting to the Society this time last year we are able to show this evening; they are still sterile, and the cancer lumps within them appear as though only just removed from the operating room.

In these experiments no antiseptics were used; the precise details of the method are described in the former paper.

We now propose to record briefly the histological appearances exhibited by sterile incubated tissues, cancerous and healthy. We had made some observations on this subject at the time our paper was read last year, but we did not think that we should be justified in bringing them before the Society until we had made further observations, and the matter still needs, indeed, much more investigation. We have during the last year incubated portions of nearly forty malignant tumours, and have made a large number of experiments with healthy tissues. These have been submitted after incubation to microscopical examination. The staining reagents employed have been logwood and fuchsin, as we found that they

[1] Towards the expenses of this research a grant was made by the British Medical Association, on the recommendation of the Scientific Grants Committee of the Association.

best show the nuclear changes, and the microscopical examinations were made with $\frac{1}{25}$ (Zeiss) water immersion, combined with Ocular 1 or 3; Reichert's $\frac{1}{15}$ homogeneous immersion, Oc. 3; Zeiss's apochromatic $\frac{1}{12}$ homogeneous immersion Oc. 8. The phenomena have been observed when the piece of cancer has been allowed to rest upon a solid medium above the drop of fluid which always collects at the lower end of the test-tube. If it soaks in the water, or if micro-organisms grow within the tube, or if the tube contain fluid nutrient jelly, the specimen becomes spoilt in a great measure for subsequent examination. The portion of cancer between its removal from the patient and its transference to the incubator must be maintained at a temperature of 100° F.

The appearances in question we have observed in both incubated and in unincubated cancer, and from numerous observations we can affirm that similar changes are not to be observed in normal tissues under similar circumstances.

These changes we may describe in detail from a portion of mammary scirrhus, after eight days' incubation, the specimen being transferred to Müller's fluid, and then to spirit, after having been in the tube for ninety days, and remaining sterile for the whole of this period. The tumour was removed on February 7th, 1887, from a widow, aged 51, whose mother had died of cancer of the breast.

The patient had had inflammation of both breasts during lactation.

Three years ago she noticed a small hard swelling at the outer border of the left breast; this gradually increased, and at the date of operation was of considerable size. The superjacent skin was adherent for about two and a half inches; tumour movable over the pectoralis. The axillary and cervical glands were diseased.

Epithelium.—The outline of the cell-protoplasm is not visible. The nuclei of the epithelial cells are very granular, the nuclear matrix between the granules being either slightly stained or colourless. In some of the nuclei there is a distinct coloured network (nucleoplasm). There are in addition distinct granules of larger size, or nucleoli. A delicate continuous outline exists to all the nuclei. Many of the nuclei have a spinous border due to the projection of the peripheral of the granules noticed, the limit of the nucleus remaining clearly defined. In other cells the granules have passed beyond the proper margin of the nucleus, and lie in the cell-protoplasm, an extremely delicate, thread-like process or stalk still connecting the granule in many instances with the rest

of the nucleus. The granules vary in size on leaving the cell-nuclei. They are mostly spheroidal, and in many instances oval, or comma-shaped, or with a more delicate filament or tail. The matrix in some of the nuclei is uncoloured, and with comparatively few darker points; in some of these nuclei one or two granules remain attached to the nucleus by a delicate process, the nucleus itself being scarcely distinguishable. The explanation of this last appearance may be that the granules have all passed out of the nucleus. Similar granules occur here and there free in the loculi.

Fibrous tissue.—The nuclei of the corpuscles are of two sizes. The smaller are more or less circular in outline, and deeply stained throughout, the nuclear matrix and nucleoplasm being in most instances indistinguishable from each other. Around the nucleus the cell-protoplasm is discernible as a narrow hazy zone. At the circumference of the nucleus are numerous projections of various sizes, some sessile, others stalked, and others free of the nucleus; these are deeply stained, and still lie in the cell-protoplasm. The larger nuclei are variously shapen, some regularly oval and of considerable size, with distinct granules and clear matrix, others narrow and of less regular form, and darkly and uniformly stained; and around these latter are particles like those first noticed. In the case of the connective-tissue corpuscles, the buds are for the most part larger and less regular in size than those described in connection with the nuclei of the epithelial cells. In the fibrous tissues around the corpuscles are minute granules, presumably arising in one or both of the above ways, and passing into the clefts of the tissue; the granules, of which the smallest are hardly visible, are fairly uniformly, and thickly distributed.

These appearances we have observed in the incubated pieces of several carcinomata (and in sarcoma) though not to the above degree.

In a second case, for example, in which a portion of scirrhus of the breast had been incubated for thirty-three days and left fifty-five days in the tube, remaining sterile throughout, the appearances observed in sections stained with logwood are as follows:

Epithelium.—Nuclei sharp, well-defined, extrusion of coloured particles, some pedunculated, others free within the granular cell-protoplasm (the outline of the cell-body being distinct), others free in the alveolus. Unstained epithelial cells occur in the groups,

in which cells no nucleus is discernible. The granules in the alveoli are often distinctly comma-shaped.

Fibrous tissue.—The corpuscles in a few spots show budding of granules from the nucleus; in the fibrous tissue in their vicinity there are in places a few free granules.

The patient from whom the preceding specimen was obtained was aged 35, married; no family history of cancer; eight children, the last being five weeks old. Twelve months ago a small hard lump was noticed at the upper and inner part of the left breast. The tumour has been growing rapidly since the last confinement. The skin was infiltrated and adherent to the new growth, adherent to pectoralis; nipple not retracted. The axillary glands were diseased.

In a **third** case the tumour was a small scirrhus from the right breast of a single woman aged 50. No family history of cancer. The growth had been noticed for fifteen months; many axillary glands were diseased. The piece of tumour was incubated for twelve days and examined after having been in the tube altogether twenty days.

Nuclei of epithelium spinous; extrusion of coloured particles into the cell-protoplasm, the limits of which are easily seen; there are a few granules free of the cells; some of the granules are distinctly comma-shaped.

The nuclei of some of the smaller connective-tissue cells show budding from their margins.

In a **fourth** the tumour, from a private patient aged about 60, was adherent to the skin, which was not ulcerated and excoriated. Axillary glands diseased.

A portion incubated for five days and kept in the tube altogether eight days, shows in fuchsin and in logwood preparations the following appearances:

Epithelium.—Nuclei lightly stained with darker points sharply defined; cell-protoplasm unstained, nebulous. From the margins of the nuclei there project granules of deeply stained substance, some attached by stalks. These granules become disconnected with the nucleus so as to be free in the cell-protoplasm. In some of the alveoli there are similar granules which appear to be free. In other places the nucleus is divided into larger irregular parts.

Fibrous tissue.—Nuclei of corpuscles very granular; budding

of coloured substance in places; a few granules free in the tissue.

Another piece of this tumour was incubated in nutrient gelatine for five days and was kept in the tube altogether sixty-three days before hardening for section. The appearances presented by this preparation are the same as those just described.

In a **fifth** case the tumour was noticed nine months previously as a hard lump in the right breast the size of a walnut; has grown rapidly during the last four months. The patient was aged 52; twelve children, three miscarriages. The breast, on admission, was infiltrated with new growth, and about the size of an orange, not adherent to pectoralis or to skin; axillary glands extensively diseased.

A piece of the tumour incubated for seven days on agar, and placed after that period in Müller's fluid, &c., shows:

Epithelium.—Cell-protoplasm with difficulty discernible in most places, nuclei very granular; the nuclei present minute sessile eminences, and here and there a stalked or capitate process of deeply stained substance, in all respects like that forming the coloured portions of the nucleus. Free granules of similar kind occur in the alveoli and in the fibrous tissue.

In a **sixth** case, a small atrophic scirrhus of the breast from a woman aged 61, the tumour had been noticed for six months; there was a history of injury. The growth was nearly central, and had led to puckering of the superjacent skin. No glands were felt enlarged and none were removed at the operation. The following appearances are observable in sections of portion of the tumour after nine days' incubation:

Epithelium.—Nuclei very granular; budding of the nucleoplasm or chromatin from the margins of several of the nuclei; some of the extruded granules have fine stalks of considerable length, three or four times the diameter of the granule. Similar granules occur in the cell-protoplasm disconnected with the nucleus, and others free in the alveoli, and in the adjacent fibrous tissue.

Fibrous tissue.—Nuclei of corpuscles very granular; here and there fine budding occurs.

In a **seventh** case the tumour was from a single woman aged 41, and was a small atrophic scirrhus of the breast, situated to the inner side of the nipple. There were two hard glands felt in the axilla.

A piece of the tumour after eight days' incubation on agar, shows, in sections stained with fuchsin, a very granular condition of the epithelial nuclei, the margins of which show in places distinct bud-like extrusions of the chromatin; free granules occur amongst the epithelial masses, and in the adjoining fibrous tissue. The nuclei of the fibrous tissue present in a few spots similar appearances.

In an **eighth** case the patient was a nurse, aged 64, married, two children. Two years previously she had received a severe blow on the right mamma, and three months after this a small tumour appeared. No family history of cancer. The skin was infiltrated; tumour not adherent to pectoralis. Many of the axillary glands were diseased.

A piece of the tumour after incubation for eight days presents (in fuchsin preparations) similar budding from the epithelial nuclei, and in a few spots disconnected chromatic granules in the cell-protoplasm.

The nuclei of the connective-tissue corpuscles show distinct budding of a similar kind.

How far exactly the process of incubation aids in producing the appearances under notice we are not quite prepared to say. That the presence of free chromatic granules in carcinoma of the breast is not limited to the incubated specimens we can assert from observation.

As an example of an ordinary mammary carcinoma we may describe a portion of the tumour previously noticed under the fifth case, the material having been prepared quite fresh, in Müller's fluid and then in spirit, and the sections stained with fuchsin, and examined under a high power of the microscope.

Epithelium.—Cell-protoplasm lightly stained; nuclei oval; intra-nuclear network faintly stained or not distinguishable; nucleoli single, or in twos or threes; the colouration of the nuclei varies in intensity.

In the fibrous tissue at several spots in the immediate vicinity of an epithelial column are small collections of deeply coloured granules of varying sizes, mostly spheroidal in shape; in some of the alveoli are a few similar granules.

In a second specimen (seventh case previously cited) chromatic granules occur in the cell-protoplasm. From a nucleus here and there are to be observed a few distinctly stalked granules or

Scirrhus mammæ put directly into Müller's fluid. *Not incubated.*

capitate processes which project into the cell-protoplasm, the border of the latter being quite distinct, polyhedral, and adapted to the adjoining epithelial cells of the column.

In a third specimen (eighth case before cited) coloured granules are to be seen in a few situations free in the alveoli and the fibrous tissue, some of them of a very delicate tailed form.

In a fourth specimen of mammary scirrhus from a widow, aged 57, the mother of three children, the disease was noticed in November, 1887, as following an injury in October; infiltrating kind; skin adherent; lymphatic glands diseased. Operation, February 4th, 1888. In the protoplasm of several of the epithelial cells occur comma-shaped granules of chromatin; and here and there epithelial cells amongst others of a group, the nuclei of which are quite devoid of coloured particles. Coloured granules are observable also in the fibrous tissue.

As an example of sarcoma experimented upon by the same method, we may adduce that of a typical spindle-celled sarcoma of the thyroid. The tumour was from a man aged 42. Repeated recurrence ensued after the first operation, and as many partial removals; the common carotid was eventually tied for hæmorrhage occurring from the growth, and the patient died April 7th, 1888. No secondary growths were found in the viscera.

In a logwood preparation made from a piece of the growth hardened in Müller's fluid and then in spirit the following appearances are seen: Nuclei of cells sharply defined, deeply

stained points of nucleoplasm in a more lightly-coloured nuclear matrix; they contain one or more nucleoli in places. Karyokinetic figures occur at rare intervals.

Leucocytes are present in fair abundance amongst the cells of the tumour; they are darkly and uniformly stained, of considerably smaller size than the nuclei of the tumour-cells, mostly spheroidal, but some are elongated. There is distinct budding from many of the latter elements, and from a few of the smaller nuclei of the tumour-cells.

There is in the section an area also of necrosis, in which no chromatic substance is seen except in the form of granules strewn in the tissue; the granules are of various shapes and sizes; some are of large size, and such are spherical; in these cases the fibrous tissue is also necrosed; in the cells bordering the disintegrating area the nuclei are unstained.

This disintegration of the nuclei is of no uncommon occurrence in portions of tumours that have undergone necrosis.

A piece after twenty-four hours' incubation on sheep's serum shows similar extrusion of the nucleoplasm of the nuclei, as described in carcinoma; here and there occur disconnected granules in the protoplasm, and free granules amongst the cells. There are no signs of degeneration in these parts.

A piece after forty-eight hours' incubation shows capitate chromatic processes projecting from a few of the nuclei into the cell-protoplasm, there being no signs of degeneration.

There is in this piece a necrotic area in which the nuclei are unstained, and the tissue thickly strewn with spherical granules of various sizes, some considerable, and derived doubtless from the nuclei of the necrosed cells.

A piece after seventy-two hours' incubation shows minute sessile buds of chromatin projecting from the margin of many of the smaller oval nuclei, there being in those parts none of the ordinary marks of degeneracy.

The appearances described we have not observed in normal tissues kept sterile on nutrient media, whether incubated at 100° F. or not. We may describe the following examples:

Muscle of a healthy dog (Plate XXXV, fig. 3), incubated eight days and subsequently hardened in Müller's fluid and then in spirit. Sections stained with fuchsin show remarkably little departure from the normal; the striation is clear in the fibres, in some the

transverse, in others the longitudinal being chiefly marked; it is not quite so sharp as in the normal. Nuclei of muscle-corpuscles oval for the most part, lightly stained plasma with darker points of matrix, not so sharply defined as the normal. Large granular plasma-cells occur in the connective tissue.

Muscle of healthy dog.—Incubated for eight days, kept in the tube altogether fifteen days; fuchsin stain. Striation indistinct, nuclei of muscle-corpuscles lightly stained with darker points so as to appear highly granular, contour regular, but less sharp than natural.

Muscle of healthy rabbit.—Kept in tube without incubation, for eight days. Transverse striation of fibres well marked; nuclei of muscle-corpuscles oval, sharply defined, granular.

Muscle of healthy rabbit.—Incubated eight days. Striation distinct in both directions; nuclei of muscle-corpuscles lightly stained with darkly coloured points, contour regular and distinct.

Muscle of healthy rabbit.—Kept without incubation for eight days and then incubated for eight days. Striation of fibres fairly well marked; nuclei of muscle-corpuscles very granular, contour of nucleus indistinguishable.

Kidney of healthy dog.—Incubated eight days. Nuclei of renal epithelium visible in some of the cells whilst in cells contiguous the nuclei are unstained; in others again the nucleus is recognisable as a very faintly stained granular spheroid. The disappearance of nucleus appears to result from the absence of coloration, the nuclear network becoming indistinguishable from the cell-protoplasm; the nuclear chromatin does not undergo dispersion; no free-coloured granules are observable in the cell-protoplasm or tissue around the tubules.

Kidney of healthy dog.—Incubated eight days, kept altogether fifteen days in the tube. Nuclei of epithelium in places well stained, in places barely visible, although in cells contiguous with the first. When stained, the nuclei are regular in form and granular; some of the nuclei are faintly stained, but wanting in sharpness of outline; in some cells no trace of nucleus is discoverable. Cell-protoplasm granular, faintly coloured.

Nuclei of connective-tissue cells of stroma well stained, granular as are also those of the capillary epithelium of the glomeruli.

Remarks.—Although we are at present unable to offer any evidence

of the real import of these remarkable appearances in cancer, we cannot help thinking that they may have an intimate bearing on the local and general infective processes of the disease.

We are acquainted with the disintegration into irregular masses of the nuclear substance which occurs in conditions of acute inflammatory necrosis, as seen in glanders, or such as is observed in the nuclei of pus-cells, or in portions of new growths in which degenerative softening has occurred, or when a tissue is subjected to unequal and excessive heating.

In the cutaneous lesions of glanders more particularly, we have observed the nuclei of the leucocytes remarkably irregular in form, divided into flask-shaped processes, mostly of relatively large size, which become detached and are found free in the surrounding tissue.

The process we have described in cancer has not, as compared with the above-mentioned cases, the appearance of being a passive cleavage or division of the nucleus ; in the epithelial cells especially the outline of the nucleus remains quite distinct and sharp, whilst the chromatic substance projects beyond.[1]

The possibility of these changes having an artificial production is of course not to be ignored, such as is known to obtain in the extrusion of the nucleus from the newt's blood-corpuscle under the influence of certain chemical agents, of the protagon from medullated nerve-fibres, and the like.

There are, however, among living processes some with which they bear comparison. One of these is the series of changes which takes place in the ovum independently of and preparatory to fertilisation.

In the maturation of the ovum there occurs an extrusion of the nuclear chromatin, with portion of the protoplasm from the cell beneath the vitelline membrane, the extruding part constituting one of the polar globules; by a repetition of this process a second globule may be extruded. The extrusion is not, according to the latest views, preceded by an ordinary karyo-kinetic process. The nucleus of the ovum, in which now no nucleoplasmic network is visible, remains as the female pronucleus, and the cell in this state is ready on fertilisation to undergo the segmentation involved in the production of an embryo. In explanation of the extension of the polar globules, Balfour has advanced the hypothesis that the

[1] Our impression is that the process occurs before the death of the tissue elements, *i. e.* within a comparatively short period after the removal of the tumour.

ovum-cell is originally hermaphrodite, and that the process implies the extrusion of the male element, its end being the prevention of parthenogenesis by necessitating the entrance of germ plasma from a second individual, the male, in order that segmentation may proceed.

The subject of spermatogenesis has been worked out in the rat from a correlated point of view, by Mr. Herbert H. Brown, of University College, in a paper in the 'Quarterly Journal of Microscopical Science,' vol. xxv, 1885. The most interesting observations in this paper relate to the so-called seminal granules, which are usually stated to arise by disintegration of the nuclei of the innermost cells of the seminiferous tubuli. According to Brown, these granules arise from the developing spermatozoa, from which they separate off during the later stages of development, and he suggests that the process may represent the elimination of the female element from the spermatozoa, the spermatozoa, like the ova, being derived from cells originally hermaphrodite.

In the process of fertilisation the head of the spermatozoon penetrates the ovum-cell, and enlarges to constitute the male pronucleus; after the fusion of the male and female pronuclei the process of segmentation commences.

It is tempting, therefore, to imagine that the appearances in carcinoma may indicate the working of a sexual process, that the extrusion of the chromatin prepares the nucleus and cell for subdivision, whilst the extruded particles may represent the male elements of such cells, and incite division in others after the manner of spermatozoa. Should this prove to be so, the extruded particles might be regarded as cancer sperm and named carcinozoa, although the latter term would hardly, under the modern limitation, include similar elements arising in sarcoma.

The only observer to whom we need refer in connection with this subject is Gussenbauer ('Zeitschrift für Heilkunde,' Band ii, 1880).

Gussenbauer's long researches were limited to the secondary infection of lymphatic glands that occurs in carcinoma, and in some cases of sarcoma. And although he does not describe or figure the remarkable appearances observed by us in the primary tumour, his conclusions with regard to gland infection are in accord with the appearances described in the present communication.

Gussenbauer states that minute granules are to be observed in

infected lymphatic glands, free amongst the cells, and also within them, his interpretation of the appearances being that the granules in question are really the seminium or infecting particles which incite the cells to multiply and take on the characters of the primary growth.[1]

It must be confessed, in criticism, that these appearances figured by Gussenbauer admit of another interpretation. It is possible that the appearance of a growth of epithelium out of elements which look like lymph-cells may be due to rapid multiplication of transported epithelium, the new and young elements being at this stage not sufficiently differentiated to admit of their being distinguished from the indifferent cells of the surrounding lymphatic tissue. And the granules mentioned by Gussenbauer, though carried by lymphatic channels from the alveoli of the carcinoma to the glands, may, in place of actively entering the cells of the latter and inciting them to an atypical division, be granules taken within the cells as are inert particles under similar circumstances. It is right to state, however, that Gussenbauer thinks that the granules, though infective, are in some cases destroyed by the lymph-cells, though the evidence which he adduces of this is not strong, viz. that clinically a temporary enlargement of glands is witnessed, independently of an inflammatory process in the primary tumour, the glandular affection not proceeding to metastatic formation.

If the granules in question have anything to do with the production of the secondary tumours it is easier to imagine this to be by inciting division in cells transported from the primary tumour to the gland than by inciting lymph-cells and the cells of the blood-vessels (Gussenbauer, loc. cit.) to undergo transformation into squamous-celled, columnar, or other epithelium.

May 15*th*, 1888.

Addendum.—The illustrations on Plate XXXIV are introduced to record the negative results obtained by incubating portions of new formations, which we recorded in the preceding volume of the Society's 'Transactions.' Fig. 1 shows a portion of a spindle-

[1] Mr. Butlin informs us that a number of sections of cancerous glands were prepared for him with great care by Mr. A. G. Francis and Mr. Lyndon, of St. Bartholomew's Hospital. The method described in the 'Zeitschrift für Heilkunde' (ii, 17) was closely followed, but he failed entirely to discover the characteristic molecules figured by Gussenbauer.

celled sarcoma of the thyroid, removed July 6th, 1887, incubated in nutrient gelatine for eight days at 100° F., and kept subsequently for some time at an ordinary room temperature. Fig. 2 shows a large piece of a mammary scirrhus, removed May 12th, 1887, incubated on agar for five days at 100° F., and then left at the ordinary temperature. At the present date (October, 1888) it has undergone not the least perceptible change; the end of the tube is hermetically sealed with gutta percha. In neither case did any growth of micro-organisms occur.

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DESCRIPTION OF PLATE XXXIV.

Illustrating Messrs. Shattock's and Ballance's note on the Histology of Cancer, &c., after Incubation.

From drawings by Mr. Shattock.

FIG. 1.—Portion of a sarcoma sterile after incubation at 100° F. in nutrient gelatine.

FIG. 2.—Portion of scirrhus of breast sterile after incubation at 100° F. on agar.

DESCRIPTION OF PLATE XXXV.

Illustrating Messrs. Shattock's and Ballance's note on the Histology of Cancer, &c., after Incubation.

From drawings by Mr. Shattock.

FIG. 1.—Section of scirrhous carcinoma of breast made from a portion incubated on solidified blood serum (sheep) for eight days at 100 F., showing the extrusion of the nuclear chromatin in the form of granules from the nuclei of the epithelial cells and connective-tissue corpuscles.

FIG. 2.—Section of healthy dog's kidney made from a portion incubated for eight days at 100° F. on solidified blood serum.

FIG. 3.—Section of healthy dog's muscle made from a portion incubated for eight days at 100° F. on beef peptone agar.

Fig. 1.

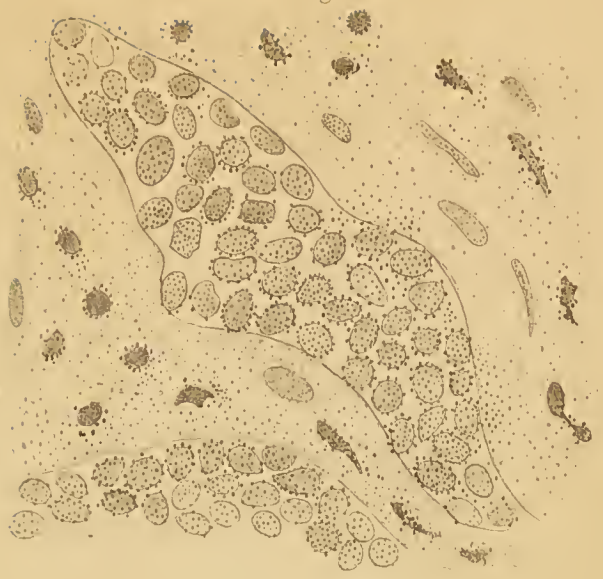

Fig 2.

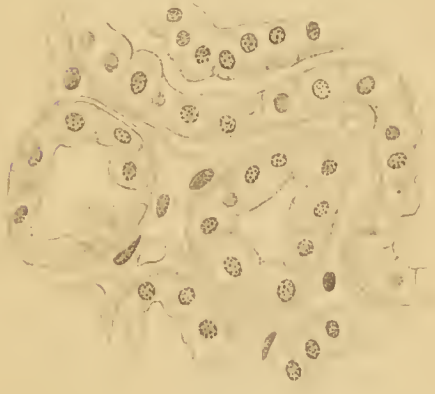

Fig. 3